REMARKS

This Amendment and Response is timely filed in response to the Office Action of November 2, 2001 because the Applicants are filing concurrently herewith a Petition for a Three-Month Extension of Time and March 2, 2002 falls on a Saturday.

In the November 2, 2001 Office Action, the Examiner subjected pending claims 87-90, 99-102 and 111-136 to restriction requirements. Claims 112 and 123-136 have been cancelled without prejudice to reassert the subject matter therein in a subsequent application, rendering the restriction requirement moot with respect to these claims. With respect to the remaining claims, the Applicants respectfully traverse the restriction requirements, particularly in light of the amended claims submitted herewith.

The Applicants thank the Examiner for the courtesy of the telephonic interviews on January 15, 2002 and January 22, 2002. During the course of these interviews, the Applicants explained that they found the restriction requirements inappropriate because all of the claims are based on the novel and unexpected finding that a human CD40+ cell transfected with a nucleic acid sequence encoding a non-human CD40 ligand, preferably a murine ligand, is capable of expressing the CD40 ligand (see p. 14, lines 10-24; p. 66, line 24 - p. 67, line 3). To determine why a human CD40+ cell cannot express human CD40 ligand, but can express the murine ligand, the Applicants investigated the structure of the nucleic acid sequences encoding human and non-human ligands that are members of the TNF family, including CD40 ligand, and found great similarity within and among species. That is, the Applicants mapped four regions or domains that are common to the nucleic acid sequences encoding both human and non-human ligands of the TNF family (see p. 29,

line 20 - end of p. 31). Moreover, the Applicants found that these domains include certain amino acid sequences that are used by post-translational mechanisms to regulate the level of expression of the ligand and/or are recognized by protease cleavage sites (see p. 35, lines 5 - 21).

Thus, the Applicants created ligand molecules, preferably human CD40 ligands, in which a domain or subdomain of the ligand is replaced with a domain or sub-domain of the same ligand from a different species, a different ligand from the same species or a different ligand from a different species (see, generally, p. 8, line 27 - p. 9, line 21; p. 32, line 1 – p. 34, line 28). The resulting human CD40 ligand is capable of being expressed by a human CD40+ cell and binding to one of its cognate receptors, preferably human CD40, thereby initiating the appropriate immune response (see p. 4, line 14 – p. 5, line 3; p. 69, line 15 - p. 82, line 9). In addition, the resulting human CD40 ligand is also less likely to be cleaved from the surface of a human cell, resulting in increased cell surface concentration of the ligand (see p. 8, lines 14-26; p. 15, lines 10-24; and p. 35, lines 5-21).

The Examiner suggested that the Applicants amend the claims to better define the invention as described above. The claims as amended are directed to several aspects of the above-described invention. Claims 87 and 88 recite a method for expressing a CD40 ligand in a human CD40+ cell by transfection of the cell with a non-human CD40 ligand, and are supported by p. 14, lines 10-24 and p. 66, line 24 - p. 76, line 3. The range of non-human ligands other than murine that is contemplated by the invention is set forth, e.g., at p. 25, line 3 - p. 26, line 14.

Claims 89 - 102 as amended recite: 1) a method for expressing a ligand capable of binding to a CD40 ligand receptor in a human CD40+ cell; and 2) a method for increasing the human cell surface concentration of a ligand capable of binding to a CD40 ligand receptor. These claimed methods are achieved by transfection of a nucleic acid sequence encoding a ligand comprising a domain or subdomain of human CD40 ligand and a domain or subdomain of non-human CD40 ligand. These claims are supported by p. 32, line 30 - p. 33, line 33; p. 36, lines 7-18; p. 82, line 11 - p. 83, line 24; and p. 83, line 25 - line 38.

Claims 103 - 107 and added claim 137 recite the same two methods as claim 89-102, but by transfection of a nucleic acid sequence encoding a ligand comprising a domain or subdomain of human CD40 ligand and a domain or subdomain of a *non-human* ligand selected from one of the recited members of the TNF family. These claims are supported by p. 34, line 29 - p. 36, line 6.

Claims 108 - 110, and added claim 138, again recite the same two methods as above, but by transfection of a nucleic acid sequence encoding a ligand comprising a domain or subdomain of human CD40 ligand and a domain or subdomain of a *human* ligand selected from one of the recited members of the TNF family. These claims are supported by p. 34, line 29 - p. 36, line 6; p. 84, line 25 and p. 85, line 25.

Thus, the Applicants respectfully traverse the first restriction requirement set forth in the November 2, 2002 action (see ¶2, p. 2 of office action). As described above, the claims that are drawn to methods of expressing CD40 ligand or increasing the cell surface concentration of CD40 ligand by transfecting cells with nucleic acid sequences encoding

murine and human CD40 ligand, as well as human CD40 ligand and another member of the TNF family, are based and dependent on the invention as recited in claim 87 -- i.e., a method for expressing a CD40 ligand in a human CD40+ cell by transfection of the cell with a nucleic acid sequence encoding a non-human CD40 ligand. With respect to the second restriction requirement of November 2, 2001 (see ¶3, p. 2), the Applicants respectfully traverse because, as described above, the claimed CD40 ligand is capable of binding to any of its cognate receptors, preferably human CD40. The claimed CD40 ligand does not have specificity for another CD40 ligand, whether murine or human. Finally, the Applicants respectfully traverse the third restriction requirement (see ¶3, p. 3) because the cells recited in claims 111 - 122 are indistinct from one another in that they are types of neoplastic cells that are capable of expressing the CD40 ligands of the present invention.

CONCLUSION

On the basis of the above, the Applicants believe withdrawal of the restriction requirements and allowance of the application are warranted, and such actions are respectfully requested. If the Examiner has any questions or comments regarding this amendment, the Examiner is respectfully urged to contact the undersigned at the number listed below.

Respectfully submitted,

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89. (Amended) A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a [chimeric CD40 ligand] domain or subdomain of human CD40 ligand and a domain or subdomain of non-human CD40 ligand into the cell.

90. (Amended) A method for increasing the concentration of a ligand on the surface of a human cell, wherein the ligand is capable of binding to a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a [chimeric CD40 ligand] domain or sub-domain of human CD40 ligand and a domain or subdomain of non-human CD40 ligand into the human cell, wherein the encoded CD40 ligand has increased stability on the surface of the cell relative to that of a human CD40 ligand.

92. (Amended) The method of claim [91] 89 or claim 90, wherein the non-human CD40 ligand domain or subdomain comprises a murine CD40 ligand domain or subdomain.

99. (Amended) The method of claim [91] 92 wherein the nucleic acid sequence [encoding the chimeric CD40 ligand] comprises SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7 or SEQ ID NO. 20.

100. (Amended) The method of claim 99 wherein the nucleic acid sequence [encoding the chimeric CD40 ligand] comprises SEQ ID NO. [3] 20.

101. (Amended) The method of claims 89 [and] or 90, wherein the introduction of the nucleic acid sequence into the cell results in induced expression of surface markers on the cell.

103. (Amended) [The method of claims 89 and 90, wherein the chimeric CD40 ligand comprises] A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or subdomain of human CD40 ligand and a domain or subdomain of a non-human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).

104. (Amended) The method of claim 103 or claim 137, wherein the non-human ligand domain or subdomain comprises a murine ligand domain or subdomain.

108. (Amended) [The method of claims 89 and 90, wherein the chimeric CD40 ligand comprises] A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or subdomain of human CD40 ligand and a domain or subdomain of a human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).

109. (Amended) The method of claim 108 or 138, wherein the [chimeric] human CD40 ligand comprises Domain IV, or a subdomain of Domain IV, of human CD40 ligand.

111. (Amended) The method of claims 89, [and] 90, 103, 108, 137 or 138, wherein the cell comprises a human neoplastic cell.

Claims 91, 112, 123 - 136 have been cancelled with this Amendment.

Claims 137 - 138 have been added with this Amendment.

Pending Claims Upon Entry of March 4, 2002 Amendment

87. A method for expressing a CD40 ligand in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a non-human CD40 ligand into the cell.

88. The method of claim 87 wherein the non-human CD40 ligand comprises murine CD40 ligand.

89. (Amended) A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or subdomain of human CD40 ligand and a domain or subdomain of non-human CD40 ligand into the cell.

90. (Amended) A method for increasing the concentration of a ligand on the surface of a human cell, wherein the ligand is capable of binding to a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or sub-domain of human CD40 ligand and a domain or subdomain of non-human CD40 ligand into the human cell, wherein the encoded CD40 ligand has increased stability on the surface of the cell relative to that of a human CD40 ligand.

92. (Amended) The method of claim 89 or claim 90, wherein the non-human CD40 ligand domain or subdomain comprises a murine CD40 ligand domain or subdomain.

93. The method of claim 92 wherein the murine CD40 ligand domain or subdomain comprises a murine CD40 ligand extracellular domain.

94. The method of claim 92 wherein the murine CD40 ligand domain or subdomain comprises Domain III, or a subdomain of Domain III, of the murine CD40 ligand.

95. The method of claim 92 wherein the murine CD40 ligand domain or subdomain comprises Domain IV, or a subdomain of Domain IV, of the murine CD40 ligand.

96. The method of claim 94 wherein the murine CD40 ligand further comprises Domain IV, or a subdomain of Domain IV, of the murine CD40 ligand.

97. The method of claim 92 wherein the murine CD40 ligand comprises Domain I, or a subdomain of Domain I, of the murine CD40 ligand.

98. The method of claim 92 wherein the murine CD40 ligand comprises Domain II, or a subdomain of Domain II, of the murine CD40 ligand.

99. (Amended) The method of claim 92 wherein the nucleic acid sequence comprises SEQ ID NO. 3, SEQ ID NO. 4, SEQ ID NO. 5, SEQ ID NO. 6, SEQ ID NO. 7 or SEQ ID NO. 20.

100. (Amended) The method of claim 99 wherein the nucleic acid sequence comprises SEQ ID NO. 20.

101. (Amended) The method of claims 89 or 90, wherein the introduction of the nucleic acid sequence into the cell results in induced expression of surface markers on the cell.

102. The method of claim 101, wherein the surface markers comprise CD54, CD80, CD86, CD58, CD70, or CD95.

103. (Amended) A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or subdomain of human CD40 ligand and a domain or subdomain of a non-human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).

104. (Amended) The method of claim 103 or claim 137, wherein the non-human ligand domain or subdomain comprises a murine ligand domain or subdomain.

105. The method of claim 104 wherein the murine ligand comprises Domain III, or a subdomain of Domain III, of the murine ligand.

106. The method of claim 104 wherein the murine ligand comprises Domain IV, or a subdomain of Domain IV, of the murine ligand.

107. The method of claim 105 wherein the murine ligand further comprises Domain IV, or a subdomain of Domain IV, of the murine ligand.

108. (Amended) A method for expressing a ligand capable of binding to a CD40 ligand receptor in a human cell that expresses a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or subdomain of human CD40 ligand and a domain or subdomain of a human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).

109. (Amended) The method of claim 108 or 138, wherein the human CD40 ligand comprises Domain IV, or a subdomain of Domain IV, of human CD40 ligand.

110. The method of claim 109 wherein the chimeric CD40 ligand comprises Domains I, II, and IV of human CD40 and Domain III of human CD70 receptor ligand.

111. The method of claims 89, 90, 103, 108, 137 or 138, wherein the cell comprises a human neoplastic cell.

113. The method of claim 111, wherein the cell comprises a neoplastic B cell.

114. The method of claim 113, wherein the neoplastic B cell comprises a CLL cell.

115. The method of claim 113 wherein the neoplastic B cell is derived from a patient with a B cell malignancy.

116. The method of claim 111 wherein the neoplastic cell comprises a T cell.

117. The method of claim 111 wherein the neoplastic cell comprises a dendritic cell.

118. The method of claim 111 wherein the neoplastic cell comprises a monocyte.

119. The method of claim 111 wherein the neoplastic cell comprises a myelomonocyte.

120. The method of claim 111 wherein the neoplastic cell comprises a cell derived from a breast tumor.

121. The method of claim 111 wherein the neoplastic cell comprises a cell derived from an ovarian tumor.

122. The method of claim 111 wherein the neoplastic cell comprises a cell derived from a lung tumor.

137. (New) A method for increasing the concentration of a ligand on the surface of a human cell, wherein the ligand is capable of binding to a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or sub-domain of human CD40 ligand and a non-human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).

138. (New) A method for increasing the concentration of a ligand on the surface of a human cell, wherein the ligand is capable of binding to a CD40 ligand receptor, comprising introducing a nucleic acid sequence encoding a domain or sub-domain of human CD40 ligand and a human ligand selected from the group consisting of CD40 ligand, TNF-alpha, TNF-beta, CD70, CD30 ligand, 4-1 BBL, nerve growth factor and TNF-related apoptosis inducing ligand (TRAIL).